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Advanced MRI in Acute Military TBI

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of the project is to test two advanced MRI methods, DTI and resting-state fMRI correlation analysis, in military TBI patients acutely after injury and correlate findings with TBI-related clinical outcomes 6-12 months later. An additional objective is to test the interaction of candidate genetic vulnerability factors with patterns of injury. The hypothesis was that these combined methods may add clinically useful predictive information following traumatic brain injury that could be of assistance in standardizing diagnostic criteria for TBI, making return-to-duty triage decisions, guiding post-injury rehabilitation, and developing novel therapeutics. The overarching hypothesis is that traumatic axonal injury, interacting with genetic vulnerability factors, is a principal cause of impaired brain function following blast-related and non-blast-related TBI.

The study was a prospective longitudinal study with subject enrollment and initial evaluation at Landstuhl Regional Medical Center in Landstuhl Germany and at 2 sites in Afghanistan. Follow-up evaluations are performed at Washington University in St Louis. Enrollment was closed June 1, 2013. Follow-up was completed on November 17, 2013. 255 subjects were enrolled at LRMC and 230 subjects were enrolled in Afghanistan. 182 subjects enrolled at LRMC and 73 subjects enrolled in Afghanistan have completed follow-up evaluations. There have been no adverse events. We have published 4 papers and have 1 final manuscript under review.

15. SUBJECT TERMS

Traumatic Brain Injury. Blast. MRI. Diffusion Tensor Imaging. Post-traumatic Stress Disorder

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Introduction

The objective of the project was to test two advanced MRI methods, DTI and resting-state fMRI correlation analysis, in military TBI patients acutely after injury and correlate findings with TBI-related clinical outcomes 6-12 months later. The interaction of candidate genetic vulnerability factors with patterns of injury was to be evaluated. The hypothesis was that these combined methods may add clinically useful predictive information following traumatic brain injury that could be of assistance in standardizing diagnostic criteria for TBI, making return-to-duty triage decisions, guiding post-injury rehabilitation, and developing novel therapeutics.
The overarching hypothesis is that traumatic axonal injury, interacting with genetic vulnerability factors, is a principal cause of impaired brain function following blast-related and non-blast-related TBI.

The specific aims of the proposal were as follows:

Aim 1) To determine whether DTI and fcMRI will noninvasively reveal abnormalities that are not present on CT or conventional MRI acutely following blast-related and non-blast-related TBI. For this aim, the goal was to enroll a total of 200 participants with TBI, 100 with blast-related injuries and 100 with non-blast-related injuries, at LRMC.

Aim 2) To assess the frequency of clinically occult traumatic axonal injury resulting from blast and non-blast mechanisms that is detectible using DTI, fcMRI, and conventional MRI. For this aim, the goal was to enroll a total of 200 participants without TBI but with other injuries at LRMC during the same 2 year period: 100 with blast-related injuries and 100 with non-blast-related injuries.

Aim 3) To use DTI and fcMRI to clarify the principal similarities and differences between blast-related TBI and TBI due to other mechanisms (e.g. motor vehicle accidents, falls, and direct blows to the head). This will be analyzed using the same 4 groups of participants described above in aims 1 and 2.

Aim 4) To test the hypothesis that specific pattern of injuries detected with these methods will predict specific longer-term neurological and neuropsychological deficits. We will collect detailed clinical information on TBI-related outcomes 6-12 months after injury at Washington University. This will include standardized neurobehavioral assessments, neuropsychological testing, and structured interviews for depression and post-traumatic stress disorder. Several pre-specified hypotheses based on known brain anatomical-clinical correlations will be tested. Also, exploratory approaches will be used as the structural bases for many post-traumatic deficits and disorders are not well understood.

Aim 5) To test the hypothesis that specific genetic factors interact with patterns of injuries to further increase the risk of specific neurological, neuropsychological, and psychiatric deficits and disorders. At follow-up, blood will be drawn for genetic testing. Genetic testing will be performed for *GABRA2* and *FKBP5* polymorphisms associated with PTSD, *5-HTTLPR* polymorphisms associated with increased risk of depression and PTSD following stressors, and *APOE* and *IL1β* genotypes associated with poor recovery from TBI.

Additional funding from DARPA supported the analysis of DTI and clinical data acquired in Afghanistan using MRI scanners installed in that country at 3 US military bases. The hypothesis guiding the studies in Afghanistan was that acute DTI abnormalities after blast-related TBI will reveal axon injury not apparent at later times, and help guide early return-to-duty decisions.

Body

In total, we published 4 papers supported by this grant.

The following table summarizes the demographic characteristics of the subjects that completed follow-up 6-12 months after enrollment.

Follow Up Participant Characteristics

Characteristic	Enrolled at LRMC				Enrolled in AFG	
	Non-blast CTL (n=69)	Blast CTL (n=27)	Non-blast TBI (n=29)	Blast TBI (n=53)	Non-blast CTL (n=34)	Blast TBI (n=38)
Age in years:						
median (range)	31 (21-49)	34 (22-46)	28.5 (20-50)	26 (19-47)	28 (19-44)	26 (20-41)
Education in years:						
median (range)	14 (9-28)	13 (10-19)	14 (9-18)	12 (12-18)	15 (12-24)	13 (12-18)
Gender no (%)						
Male	63 (91%)	25 (92%)	26 (87%)	51 (96%)	27 (79%)	36 (95%)
Female	6 (9%)	2 (8%)	3 (13%)	2 (4%)	7 (21%)	2 (5%)
Race/ethnicity no (%)						
White	50 (73%)	20 (77%)	19 (60%)	40 (76%)	22 (65%)	29 (77%)
African American	16 (23%)	4 (12%)	7 (27%)	4 (6%)	5 (15%)	2 (5%)
Hispanic/Latino	3 (4%)	2 (8%)	3 (10%)	7 (14%)	7 (20%)	7 (18%)
Asian	0	1 (3%)	1 (3%)	2 (4%)	0	0
Branch of Service no (%)						
US Army	55 (80%)	24 (89%)	26 (90%)	46 (90%)	13 (38%)	32 (84%)
US Air Force	11 (16%)	0	2 (7%)	1 (2%)	2 (6%)	0
US Marine Corps	3 (4%)	3 (11%)	1 (3%)	5 (6%)	3 (9%)	6 (16%)
US Navy	0	0	0	1 (2%)	16 (47%)	0
Military Rank no (%)						
Enlisted	63 (91%)	24 (89%)	27 (93%)	52 (98%)	24 (71%)	35 (92%)
Officer	6 (9%)	3 (11%)	2 (7%)	1 (2%)	10 (29%)	3 (8%)
Theatre of Operation no (%)						
Afghanistan	55 (80%)	21 (77%)	18 (60%)	50 (94%)	34 (100%)	38(100%)
Iraq	14 (20%)	6 (23%)	11 (40%)	3 (6%)	0	0

In the first publication (Mac Donald et al. 2014a), we defined the functional outcomes following blast-related TBI in military personnel enrolled in earlier cohorts, supported by the closed PT075299 award. To summarize, moderate overall disability in 41/47 (87%) blast-plus TBI subjects and a substantial but smaller number (11/18, 61%, $p = 0.018$) of demographically similar US military controls without TBI evacuated for other medical reasons. Cognitive function assessed with a neuropsychological test battery was not different between blast-plus TBI subjects and controls; performance of both groups was generally in the normal range. No subject was found to have focal neurological deficits. However, 29/47 (57%) of blast-plus subjects with TBI met all criteria for post-traumatic stress disorder (PTSD) versus 5/18 (28%) of controls ($p = 0.014$). PTSD was highly associated with overall disability; 31/34 patients with PTSD versus 19/31 patients who did not meet full PTSD criteria had moderate to severe disability ($p = 0.0003$). Symptoms of depression were also more severe in the TBI group ($p = 0.05$), and highly correlated with PTSD severity ($r = 0.86$, $p < 0.0001$).

In the second publication (Mac Donald et al. 2014b), we directly compared clinical outcomes in military personnel with blast-related TBI vs nonblast-related TBI. This work was fully supported by the PT090444 award. To summarize, global outcomes, headache severity, neuropsychological performance, and surprisingly even PTSD severity and depression were indistinguishable between the two TBI groups, independent of mechanism of injury. Both TBI groups had higher rates of moderate to severe overall disability than the respective control groups: 41/53 (77%) of blast plus impact TBI and 23/29 (79%) of nonblast TBI vs. 16/27 (59%) of blast-exposed controls and 28/69 (41%) of non-blast-exposed controls. In addition, blast-exposed controls had worse headaches and more severe PTSD than non-blast-exposed controls. Self-reported combat exposure intensity was higher in the blast plus impact TBI group than in nonblast TBI group and was higher in blast-exposed controls than in non-blast-exposed controls. However, combat exposure intensity did not correlate with PTSD severity in the TBI groups, but a modest positive correlation was observed in the controls. Overall outcomes were most strongly correlated with depression, headache severity, and number of abnormalities on neuropsychological testing. However a substantial fraction of the variance in overall outcome was not explained by any of the assessed measures.

In the third publication (Adam et al. 2015), we assessed US Military subjects enrolled and scanned at 2 sites in Afghanistan. The objective of the study was to evaluate whether diffusion tensor imaging (DTI) will noninvasively reveal white matter changes not present on conventional MRI in acute blast-related mTBI and to determine correlations with clinical measures and recovery. We performed a prospective observational study of 95 mTBI and 101 healthy control US military service members enrolled within 7 days from injury in Afghanistan. Assessments included Rivermead Post-Concussive Symptom Questionnaire (RPCSQ), Post-Traumatic Stress Disorder Checklist Military (PCLM), Beck Depression Inventory (BDI), Balance Error Scoring System (BESS), Automated Neurocognitive Assessment Metric (ANAM), conventional MRI and DTI. We found significantly greater impairment was observed in mTBI participants versus controls: RPCSQ (19.7±12.9 vs. 3.6±7.1, $p<0.001$), PCLM (32±13.2 vs. 20.9±7.1, $p<0.001$), BDI (7.4±6.8 vs. 2.5±4.9, $p<0.001$), and BESS (18.2±8.4 vs. 15.1±8.3, $p=0.01$). The largest effect size in ANAM performance decline was in simple reaction time (mTBI 74.5±148.4 vs. control -11±46.6 ms, $p<0.001$). Fractional anisotropy was significantly reduced in mTBI compared to controls in the right superior longitudinal fasciculus (0.393±0.022 vs. 0.405±0.023, $p<0.001$). No abnormalities were detected with conventional MRI. Time to return-to-duty correlated with RPCSQ ($r=0.53$, $p<0.001$), ANAM simple reaction time decline ($r=0.49$, $p<0.0001$), PCLM ($r=0.47$, $p<0.0001$), and BDI ($r=0.36$ $p=0.0005$). Thus, in conclusion, somatic, behavioral and cognitive symptoms and performance deficits are substantially elevated in acute blast-related mTBI. Post-concussive symptoms and performance on measures of post-traumatic stress disorder, depression and neurocognitive performance at initial presentation correlate with return-to-duty time. Although changes in Fractional Anisotropy are uncommon and subtle, DTI is more sensitive than conventional MRI in imaging white matter integrity in blast-related mTBI acutely.

In the fourth publication (Mac Donald et al. 2015), we evaluated 6-12 month outcomes in US Military subjects enrolled in Afghanistan. To summarize, high rates of adverse outcomes have been reported following blast-related concussive traumatic brain injury (TBI) in US Military personnel, but the extent to which such adverse outcomes can be predicted acutely after injury is unknown. We performed a prospective, observational study of US Military personnel with blast-related concussive TBI (n=38) and controls (n=34) enrolled between March and September 2012. Importantly all subjects returned to duty and did not require evacuation. Subjects were evaluated acutely 0-7 days after injury at two sites in Afghanistan and again 6-12 months later in the United States. Acute assessments revealed heightened post-concussive, post-traumatic stress, and depressive symptoms along with worse cognitive performance in TBI subjects. At 6-12 month follow up, 63% of TBI subjects and 20% of controls had moderate overall disability. TBI subjects showed more severe neurobehavioral, post-

traumatic stress, and depression symptoms along with more frequent cognitive performance deficits and more substantial headache impairment than controls. Logistic regression modeling utilizing only acute measures identified that a diagnosis of TBI, older age, and more severe post-traumatic stress symptoms provided a good prediction of later adverse global outcomes (area under the receiver-operating characteristic curve = 0.84). Thus, US military personnel with concussive blast-related TBI in Afghanistan who returned to duty still fared quite poorly on many clinical outcome measures 6-12 months following injury. Poor global outcome appears to be largely driven by psychological health measures, age, and TBI status. The effects of early interventions and longer term implications of these findings are unknown.

For the final manuscript (Mac Donald et al, currently under review at the Journal of Clinical Investigation), we analyzed the combined the data sets from subjects enrolled between 2008 and 2013, supported by both the PT07 and PT09 grants. We recognized that care for US Military personnel with combat-related concussive traumatic brain injury (TBI) has substantially changed over the years during which we were performing our studies, yet trends in clinical outcomes remain largely unknown. We analyzed data from 321 active-duty US Military personnel enrolled from 2008-2013 at Landstuhl Regional Medical Center in Germany and 2 sites in Afghanistan who sustained concussive TBI in theater along with 254 Military controls. We prospectively assessed clinical outcomes 6-12 months later in 199 with concussive TBI and 148 controls. We found that global disability, neurobehavioral impairment, depression severity, and post-traumatic stress disorder (PTSD) severity were worse in concussive TBI groups in comparison to controls in all cohorts. Global disability primarily reflected a combination of work-related and non-work-related disability. There was a decrease over time of 5.9 points out of 136 possible on the Clinician Administered PTSD Scale (-4.3%) per year (95% confidence interval 2.8 to 9.0 points, $p=0.0037$ linear regression, $p=0.03$ including covariates in generalized linear model). No other significant trends in outcomes were found. Global disability was more common in those with TBI, those evacuated from theater, and those with more severe depression and PTSD. Disability was not significantly related to neuropsychological performance, age, education, self-reported sleep deprivation, injury mechanism or date of enrollment. We concluded that across multiple cohorts of US Military personnel with combat-related concussion, 6-12 month outcomes have improved only modestly and are often poor. Future focus on early depression and PTSD after concussive TBI appears warranted. However, additional studies will be required to fully address the root causes of persistent disability after wartime injury.

The complete submitted manuscript is attached.

There were two substantial technical challenges:

1) The quality of the MRI scans from the 3T scanner in LRMC was not as good as originally hoped. We worked in collaboration with Dr. Carlo Pierpaoli at NIH and researchers at WashU to attempt to correct some of the signal distortions present in the scans. This is still ongoing, with support from separate funds. Importantly, the entire field of advanced MRI research has become much more attuned to data quality issues (Jones et al. 2013). For subsequent studies, we are putting together a set of up-front quality control metrics that will ensure that good quality data is obtained from the beginning of the project so that these issues can be minimized. Specifically, we will ensure that
    a. signal to noise is >25 for all regions of interest including the orbitofrontal regions that are vulnerable to susceptibility artifact,
    b. test-retest reliability on the same normal subject is >95% in all regions of interest,
    c. Gibbs ringing is not present,
    d. field of view includes the whole brain including brainstem,
    e. subject motion is minimized using head coil padding and a nose bridge.
    f. Eddy current distortions are corrected by obtaining 2 sets of images with opposite phase encoding directions.
    g. Multiple b-zero images are acquired to reduce noise in mean diffusivity measurements.

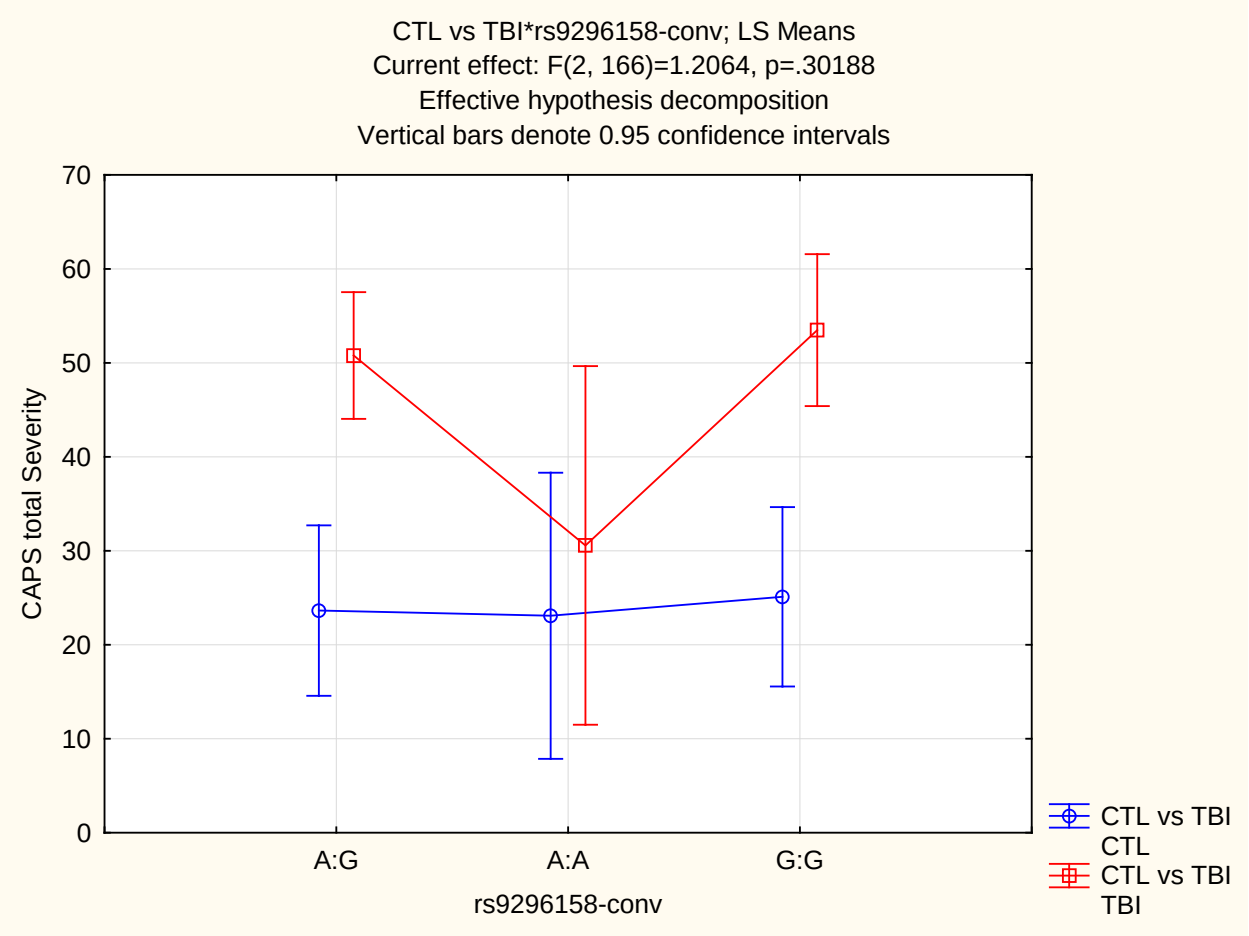
Ongoing work supported by other funds:

Dr. Laurena Holleran (post-doc, Brody lab) is developing advanced diffusion MRI methods to improve quality of imaging in brain regions vulnerable to TBI, especially orbitofrontal and temporal pole regions. This imaging uses scanners with very high gradient strength such as those developed for the Human Connectome Project (Sotiropoulos et al. 2013). This approach has tremendous promise for future TBI studies.

Dr. Kihwan Han (now at UT Dallas) is continuing to analyze resting state functional connectivity data (Han et al. 2013) from the cohort enrolled at LRMC. This has been challenging due to the same imaging quality issues arising with the DTI data.

Dr. Christine Mac Donald (now at U Washington) is continuing to analyze clinical data. She successfully obtained 2 grants (CENC subaward and NIH R01) to perform 5-7 year follow-up evaluations on the subjects enrolled at LRMC and Afghanistan. To our knowledge, this will be the first longer-term longitudinal outcome study of US military personnel with blast-related TBI from the wars in Iraq and Afghanistan.

2) The number of subjects with complete follow-up has proven to be too small to perform genetic analyses with sufficient statistical power in these mixed ancestry population. Our initial finding that polymorphisms in the FKBP5 allele appeared to influence PTSD severity (CAPS) following TBI were not confirmed with analysis of additional subjects. There were too few subjects with the rare AA allele which we had hypothesized could be protective from PTSD following TBI (note the large error bars). Our plan is to combine our data with those from other cohorts around the country to improve statistical power. We have discussed this with Dr. Kerry Ressler at Emory University, one of the worlds' leaders the genetics of PTSD in civilian populations and the discoverer of the FKBP5 effect in civilian PTSD (Binder et al. 2008).


CTL vs TBI*rs9296158-conv; LS Means
Current effect: F(2, 166)=1.2064, p=.30188
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
CAPS total Severity
70
60
50
40
30
20
10
0
A:G
A:A
G:G
rs9296158-conv
CTL vs TBI
CTL
CTL vs TBI
TBI

There were no adverse events. One subject expressed suicidal ideation during the evaluation at WashU. This was handled per protocol and did not result in an adverse events.

Note to File for TB5634W
11 November 2013
The subject was seen as part of our research study on 10 November 2013. SM endorses a recent suicide attempt followed by hospitalization for 10 days and subsequent release on 5 November 2013. Significant signs of depression were noted included car repossession, power and water being shut off due to the SM's in ability to pay his bills and general apathy towards life. He recounts he stopped showering, stopped eating, and then overdosed on prescription medication as a way to "Fix all of his problems". Pysch evaluation by LCSW Justin Hampton noted severe Depression, and moderately severe PTSD. Study Director Christine Mac Donald saw the SM for neurobehavioral exam. Following the exam, Dr. Mac Donald met privately with his wife to discuss the concerns raised regarding his mental health and safety. Wife denied any active intent as did the SM but both stated that he had attempted suicide prior. Wife expressed considerable stress and feelings of pressure and responsibility for his safety. Dr. Mac Donald gave the wife a list of resources that she could use to find help for both of them local to their area and confirmed with the wife that there was a plan for continued care. SM and wife both independently mentioned that he has an appointment in a month to follow up with a mental health provider although they did not know the person's name or who the case would be assigned to. Both Mr. Hampton and Dr. Mac Donald confirmed in their respective sessions that the SM has a safety plan given to him upon his release and both offered to assist him in finding additional resources local to his area.
Per protocol, Study Director, Dr. Mac Donald followed up with our onsite Psychiatrist Dr. Elliot Nelson to brief him on the case and confirm that proper action was taken. Dr. Nelson was briefed at 0805 Monday 11 November 2013. Since the SM denies active suicidal intent and speaks of 'having a reason to live' with his wife back in the picture, no immediate action on the part of the study advised by Dr Nelson. Dr. Nelson agreed it was sufficient to provide and suggest resources to both the wife and SM since continued care has already been planned following his release. The SM does have a history of alcoholism, what appears to be major depression, previous suicide attempts, and poor family history that we believe put him at high risk of further harm.
This document is intended to serve as an official note of the actions taken by the research study regarding this case.

PI: I concur. No evidence of harm.

Key Research Accomplishments:

Completed follow-up evaluations
Published 4 original research papers
Submitted 1 additional manuscript

Reportable Outcomes from the Current Project:

Publications:

1. CL Mac Donald, AM. Johnson, L Wierzechowski, E Kassner, T Stewart, EC Nelson, NJ Werner, D Zonies, J Oh, R Fang, **DL Brody** "Prospectively Assessed Clinical Outcomes in Concussive Blast vs. Non-blast Traumatic Brain Injury in Evacuated US Military Personnel." JAMA Neurology; 71(8):994-1002 (2014). doi: 10.1001/jamaneurol.2014.1114
2. CL Mac Donald, AM Johnson, EC Nelson, NJ Werner, R Fang, S Flaherty and **DL Brody.** "Functional Status Following Blast-Plus-Impact Complex Concussive Traumatic Brain Injury in Evacuated United States Military Personnel." Journal of Neurotrauma. 31: 889-98 (2014).
3. Adam, O., Mac Donald, C. L., Rivet, D., Ritter, J., May, T., Barefield, M., Duckworth, J., LaBarge, D., Asher, D., Drinkwine, B., Woods, Y., Connor, M. and Brody, D. L. (2015). "Clinical and imaging assessment of acute combat mild traumatic brain injury in Afghanistan." Neurology **85**(3): 219-227. http://www.ncbi.nlm.nih.gov/pubmed/26109715
4. Mac Donald, C. L., Adam, O. R., Johnson, A. M., Nelson, E. C., Werner, N. J., Rivet, D. J. and Brody, D. L. (2015). "Acute post-traumatic stress symptoms and age predict outcome in military blast concussion." Brain **138**(Pt 5): 1314-1326. http://www.ncbi.nlm.nih.gov/pubmed/25740219

Abstracts and Presentations:

The PI and Dr. Mac Donald presented aspects of the results at several meetings and seminars:
2014 Virginia Commonwealth University,
2014 Military Neuroimaging Review, Ft Dietrich
2014 MHSRS
2014 University of Kentucky
2014 Massachusetts General Hospital
2014 University of Pittsburgh
2013 MHSRS meeting.
2013 Workshop on Genetic Disease Models of Psychiatric and Neurological Diseases, Utrecht, NL
2013 American Society for Neural Therapy and Repair, Clearwater, FL
2013 Academy of Sciences, St Louis
2013 Toronto Sick Kids Head Injury in Sport meeting
2013 Johns Hopkins University TBI conference
2012 ATACCC meeting.
2012 Research Seminar at Baylor College of Medicine
2012 National Neurotrauma Society Meeting
2012 Research Seminar at Wayne State
2012 Research Seminar at Loma Linda
2012 Research Seminar at University of Missouri, Columbia
2012 Neurology Grand Rounds at the University of Pennsylvania
2011 Society for Neuroscience Meeting
2011 ATACCC meeting. (The PI won the first prize award for poster presentation)
2011 Research Seminar at The Ospedale Maggiore Policlinico, University of Milan, Italy
2011 National Neurotrauma Society Meeting

2011 International Society for Magnetic Resonance in Medicine (ISMRM) meeting.
2011 International Neurotrauma Society Meeting
2011 Safar Symposium, University of Pittsburgh
2011 MIT Blast-injury Modeling Symposium
2010 Army Vice Chief of Staff Blue Ribbon Symposium on TBI and PTSD
2010 Society for Neuroscience Meeting

Conclusion:

The project resulted in a productive line of investigation. There were several technical challenges which will be addressed in future studies.

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Appendices:

1. CL Mac Donald, AM Johnson, L Wierzechowski, E Kassner, T Stewart, EC Nelson, NJ. Werner, O Adam, D Rivet, S Flaherty, J Oh , D Zonies, R Fang, and DL Brody "Outcome Trends Following US Military Concussive Traumatic Brain Injury" *(under review)*

TITLE PAGE

Full Title: Outcome Trends Following US Military Concussive Traumatic Brain Injury

Short Title: Outcome Trends Following US Military Concussive TBI

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ABSTRACT:

Background: Care for US Military personnel with combat-related concussive traumatic brain injury (TBI) has substantially changed in recent years, yet trends in clinical outcomes remain largely unknown.

Methods: We enrolled 321 active-duty US Military personnel from 2008-2013 at Landstuhl Regional Medical Center in Germany and 2 sites in Afghanistan who sustained concussive TBI in theater along with 254 Military controls. We prospectively assessed clinical outcomes 6-12 months later in 199 with concussive TBI and 148 controls.

Results: Global disability, neurobehavioral impairment, depression severity, and post-traumatic stress disorder (PTSD) severity were worse in concussive TBI groups in comparison to controls in all cohorts. Global disability primarily reflected a combination of work-related and non-work-related disability. There was a decrease over time of 5.9 points out of 136 possible on the Clinician Administered PTSD Scale (-4.3%) per year (95% confidence interval 2.8 to 9.0 points, p=0.0037 linear regression, p=0.03 including covariates in generalized linear model). No other significant trends in outcomes were found. Global disability was more common in those with TBI, those evacuated from theater, and those with more severe depression and PTSD. Disability was not significantly related to neuropsychological performance, age, education, self-reported sleep deprivation, injury mechanism or date of enrollment.

Conclusion: Across multiple cohorts of US Military personnel with combat-related concussion, 6-12 month outcomes have improved only modestly and are often poor. Future focus on early depression and PTSD after concussive TBI appears warranted. However, additional studies will be required to fully address the root causes of persistent disability after wartime injury.

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INTRODUCTION

There are more than 2 million US military veterans of the recent conflicts in Iraq and Afghanistan.(1) It is estimated that 19% of this deployed force suffered a possible traumatic brain injury (TBI) in these wars(2). Of clinician diagnosed TBIs in across both deployed and non-deployed US military personnel, 82.5% have been classified as mild TBI or concussion (1, 3). The long term clinical impact of these war time injuries remains incompletely described (4, 5). Most previous studies in active-duty US military personnel and veterans have been restricted to single cohort evaluations(6-15) often involving retrospective record review (6-8) or self-report(9-13, 15, 16).

As part of our efforts to assess the role of advanced MRI methods in the identification and assessment of the effects of concussive TBI in US military personnel(17, 18) we obtained standardized, prospective, clinician rating-based outcome information 6-12 months after injury in four distinct cohorts of US Military personnel between 2008 and 2013 using essentially identical methods across studies(19-21). This provided the opportunity to assess for trends in outcome over time. Our overarching goal was to analyze data across these cohorts to determine the relationship between global disability and clinical measures including neurobehavioral symptoms, neuropsychological performance, and psychiatric symptomatology.

During the course of our studies, the US Military issued a Directive Type Memorandum (DTM 09-033) on June 21, 2010 with the objective to "identify, track and ensure the appropriate protection of service members exposed to potential concussive events, including blast events, to the maximum extent possible."(22). Prior to June 2010, TBI screening was not routinely implemented in Afghanistan or Iraq and there were no standardized provisions for recurrent TBI prevention or treatment. Return-to-duty decisions were generally left to line commanders, not medical providers. Thus, many injuries were not immediately reported(2). We therefore also used our data to compare clinical outcomes following concussive brain injury in military personnel injured in combat treated before and after the issuance of the DTM, though this was not a pre-specified goal of our research studies.

RESULTS

Demographics of the subjects were consistent across all four cohorts from 2008-2013. Most subjects were young, high-school educated, male, enlisted members of the US Army (**Table 1**). In addition, demographics were consistent within groups comparing those who completed follow up at 6-12 months and those who did not (**Table 2-3**).

Scores on the Military Acute Concussion Evaluation (MACE) completed after medical evacuation to LRMC or directly following injury in Afghanistan did not significantly differ across studies within concussive TBI groups (**Fig. 2A**, p=0.87 Kruskal Wallis ANOVA). Furthermore, there were no trends in MACE as a function of date of injury (**Fig. 2B**, p=0.52 linear regression).

Global Outcomes

Global outcomes at 6-12 month follow-up assessed using the Glasgow Outcome Scale-Extended significantly differed by group (**Fig. 3A**, $p<0.0001$ Kruskal-Wallis ANOVA). Concussive TBI subjects had significantly worse outcomes than both the non-blast control subjects ($p<0.0001$) and blast controls ($p<0.0001$, one-sided Mann-Whitney U tests). The blast control subjects exhibited significantly worse outcomes than non-blast control subjects (p=0.0044, two-sided Mann-Whitney U). The percentage of subjects who had an overall outcome of moderate to severe disability ranged from 62-96% in the TBI cohorts.

For most cohorts, the majority (70-82%) of injured subjects with moderate disability had disability due to a combination of work and non-work factors (**Fig. 3B**). The exception was for the most recent study cohort involving subjects enrolled in Afghanistan (Study 4) in which 52% of those with moderate disability had non-work disability only. A minority of injured subjects (6-12%) had work-related disability only.

Neurobehavioral Assessment

Neurobehavioral impairment assessed using the Neurobehavioral Rating Scale-Revised also differed significantly by group (**Figure 4A**, $p<0.0001$ Kruskal Wallis ANOVA). Concussive TBI subjects exhibited

significantly worse neurobehavioral impairments than both non-blast controls ($p<0.0001$) and blast controls ($p=0.001$, one-sided Mann-Whitney U tests). Blast controls were more impaired than non-blast controls ($p<0.0001$, two-sided Mann-Whitney U test). Impairments were noted in each of the 5 sub-domains: mood/affect, executive/cognitive function, oral/motor function, positive symptoms, and negative symptoms (**Fig. 4B-F**; all $p<0.0001$, Kruskal Wallis ANOVA).

Neurobehavioral impairments among concussive TBI subjects were less severe for those injured after June 21, 2010 than for those injured before the issuance of the DTM (**Figure 4G**, $p=0.017$, Mann Whitney U test). The significance was marginal ($p=0.057$, ANCOVA) when including the following covariates: age, education, branch (Army vs. other), race (white vs. other), mechanism of injury (blast vs. non-blast) and evacuation to LRMC vs. treatment in Afghanistan with return to duty. None of the covariates individually were significantly associated with neurobehavioral impairment. Furthermore, there was a trend towards less severe neurobehavioral impairment after concussive TBI as a function of date of injury (**Figure 4H**). Average impairments decreased by 1.1 points out of 87 per year (95% confidence interval from 0.4 to 1.8 points) from 2008-2013 ($r^2=0.04$, $p=0.0037$, linear regression). However, this trend lost statistical significance when including the covariates in the statistical model ($p=0.08$, generalized linear model).

Neuropsychological Testing

Across cohorts, concussive TBI groups generally performed similarly to controls on neuropsychological testing (**Table 4**). Evaluation at the single-subject level revealed subsets of concussive TBI subjects with impaired neuropsychological performance (**Fig. 5**). Abnormal performance on each individual assessment was defined as a subject's score that fell outside 2 standard deviations worse than the mean of the pooled non-blast control group for that exam. For each subject, the number of tests with abnormal performance was then summed. The number of subjects per group was then compared to what would be expected by chance. More subjects with abnormal test performance in 2 or more neuropsychological assessments than expected by chance were observed in the evacuated TBI subjects from studies 1-3 (51/161, 31%, $p=0.00001$), the non-evacuated

TBI subjects from study 4 (10/38, 26%, p=0.003), and blast control subjects (10/45. 22%, p=0.01, Chi-square tests). There were no differences between subjects injured before vs. after the issuance of the DTM (p=0.87) and no trends in neuropsychological test abnormalities after concussive TBI as a function of date of injury (p=0.53).

Performance on three tests was significantly different across studies by Kruskal Wallis ANOVA after correction for multiple comparisons. This included a timed 25 foot walk (p=0.0001); the 25 hole grooved pegboard test (p=0.00001), an assessment of upper extremity motor speed and coordination; and the Controlled Oral Word Association test (p=0.001), an assessment of verbal fluency. For each assessment the non-blast control subjects from studies 3 and 4 outperformed blast control subjects and the medically evacuated concussive TBI groups from studies 1-3. There were no significant differences after Dunn's correction for multiple comparisons between the non-blast controls and non-medically evacuated concussive TBI group from study 4. Likewise, there were no significant differences between blast controls and concussive TBI groups.

Post-Traumatic Stress Disorder and Depression

Clinician ratings of depression and PTSD severity substantially differed across groups (**Fig. 6,** p<0.0001, Kruskal Wallis ANOVA). Concussive TBI subjects were more depressed than both non-blast control (p<0.0001) and blast control (p=0.0062, one-tailed Mann-Whitney U tests) subjects. Blast controls also had more depression than non-blast controls (p=0.0007, two-tailed Mann-Whitney U test). Similarly, concussive TBI subjects also had more severe PTSD than both non-blast controls (p<0.0001) and blast controls (p=0.0004, one-tailed Student's t tests). Blast controls also had more severe PTSD than non-blast controls (p<0.0001 two-tailed Student's t test). All three PTSD domain sub-scores (re-experiencing, avoidance and numbing, hyperarousal) were found to also be significantly different across groups, as was self-reported sleep deprivation (**Fig. 7**, p<0.0001 Kruskal-Wallis ANOVAs).

For the poor sleep index, the concussive TBI groups were not collapsed because blast TBI subjects from study 1 differed significantly from blast TBI subjects in study 4 (p<0.05, Dunn's Multiple Comparison Test).

However, both non-blast control groups were pooled and both blast control groups were pooled because these did not differ from each other. With this pooling, the overall ANOVA was again significant ($p<0.0001$). In post-hoc testing, blast+impact concussive TBI subjects from studies 1 and 2 had higher poor sleep indexes than non-blast controls ($p<0.05$) but none of the TBI groups differed from the blast controls. The blast control group was not statistically significantly different from the non-blast control group.

Among concussive TBI subjects, both depression and PTSD were less severe for those injured after the issuance of the DTM than before (**Fig. 6C-D**, p=0.02 for depression p=0.006 for PTSD, Mann Whitney U tests). However, the statistical significance was lost (p=0.12 for depression, p=0.07 for PTSD, ANCOVA) when including the covariates. Evacuated TBI subjects (studies 1-3) had more severe PTSD than non-evacuated (study 4) subjects (p=0.03) in this analysis (**Fig. 6B**). There were trends towards less severe depression and PTSD as a function of date of injury (**Fig. 6E-F**). Depression decreased by 1.6 points (95% confidence interval 0.4 to 2.8 points) out of 60 and PTSD decreased by 5.9 points (95% confidence interval 2.8 to 9.0 points) out of 136 (-4.3%) on average per year from 2008-2013 (r^2=0.035, p=0.012 for depression, r^2=0.069, p=0.00037 for PTSD, linear regression). The trend for depression lost significance (p=0.15) but the trend for PTSD maintained statistical significance when including the covariates (p=0.03, generalized linear models).

Among blast controls, there were no differences in depression (p=0.59) or PTSD (p=0.42) for those enrolled after vs. before the issuance of the DTM. Likewise, there were no trends in depression (r^2= 0.0087, p=0.52) or PTSD (r^2= 0.003, p=0.72) as a function of date of first evaluation.

Multivariate Correlates of Dichotomized Global Outcome

Dichotomized global outcome was defined as follows: GOS-E scores of 7-8 were categorized as good outcome and scores of ≤6 were defined as disabled. Candidate variables for logistic regression modeling included PTSD severity (CAPS total score), depression severity (MADRS), self-reported sleep deprivation, group distinction (Control vs. TBI), exposure (blast vs. non-blast), enrollment site distinction (evacuated vs. non- evacuated), age, education, number of neuropsychological test abnormalities, date of enrollment, and

enrollment before vs. after the issuance of the DTM. The best logistic regression contained the CAPS, MADRS, group distinction (control vs. TBI), and enrollment site distinction (evacuated vs. non-evacuated) with a receiver-operating characteristic area under the curve of 0.8351 (**Fig. 8**). Higher likelihood of disability was observed in service members with diagnoses of concussive TBI, those who were evacuated and those who had more severe PTSD and depression. Date of enrollment and enrollment before vs. after the issuance of the DTM did not contribute to the best model of global outcome.

DISCUSSION

In summary, there were adverse clinical outcomes 6-12 months after concussive TBI in a substantial majority of US military personnel injured in theater. Outcomes were generally consistent across four cohorts enrolled from 2008-2013, though there were modest improvements in PTSD severity over time. Blast-exposed service members without apparent TBI had outcomes that were intermediate between subjects with concussive TBI and non-blast-exposed military controls. Adverse global outcomes were typically in both work and non-work related domains. Overall disability was most strongly associated with concussive TBI diagnosis, PTSD and depression severity, and requirement for medical evacuation from theater.

The percentage of concussive TBI subjects with poor overall outcome at 6-12 months (62-96%) far exceeds what is routinely reported in the civilian literature for concussive TBI patient populations even with polytrauma where reports range from 22-47%(23, 24). Blast-related mechanisms causing TBI do not appear to be a major contributor, as subjects with non-blast-related TBI fared comparably.(20, 21)

This is the first study to our knowledge compare outcomes before and after issuance of the Department of Defense DTM 09-033 in 2010 regarding identification and treatment of military concussive TBI in theater(22). While such an evaluation was not our pre-specified purpose, these 4 longitudinal cohorts assessed in a homogenous fashion over 5 years provided a serendipitous opportunity to do so. However, our data in no

way reflect the efficacy of the DTM with regard to its stated purpose and should not be interpreted as such. Furthermore, none of our data directly bear on the question of the extent to which the specific provisions in the DTM were actually followed.

The results from our study fit well with those of the recently reported prospective longitudinal Marine Resiliency Study.(25) In the Marine Resiliency Study, subjects with deployment-related TBI had increased PTSD severity 3 months after deployment, especially in participants with less severe pre-deployment PTSD symptoms. However, global disability was not reported in the Marine Resiliency Study. Although dates of enrollment spanned 2008-2012, analysis of outcomes as a function of time were not presented.

One of the most striking findings in this report is that over a 5 year period from 2008 to 2013, the severity of disability, PTSD and depression following concussive TBI in deployed US military personnel improved only marginally. A reasonable conclusion from our result could be that more effective interventions to treat PTSD and depression in this setting should be considered a top priority. Such interventions were not a part of DTM, although an assessment acute stress disorder is a mandatory element of the comprehensive neurological evaluation performed in those who have had 3 or more documented concussions within a 12 month period. Pre-injury resilience training and interventions starting at very early times following concussive TBI in high risk individuals, such as military service members, could be effective strategies. The extent to which the specific act of medical evacuation which caused the service members to leave the support of their units contributed to more severe PTSD and depression remains to be determined.

In other contexts, both PTSD and depression are at least partially treatable with a combination of medications(26-29) and psychological interventions such as prolonged exposure(30-32) or cognitive processing therapy (31, 33). No additional clinical care was provided as part of these research studies and we did not collect data on the specific interventions the study participants received. However, recent literature indicates that only a relatively small fraction of US military service members complete a full course of treatment for PTSD and depression. Reasons cited include lack of access, fear of stigma, poor follow-up compliance, and

initial worsening of symptoms during the early part of the therapy. Likewise, reasons for less than ideal pharmacotherapy effectiveness include troubling side effects, irregular compliance, and concomitant drug or alcohol use(2, 29, 34-38). Anecdotal reports obtained from the participants in our cohorts are in line with the above cited concerns. Alternatively it is possible that the effects of these standard treatments for PTSD and depression are less effective in the context of TBI because of brain circuitry disruption and neurochemical deregulation. Thus, based on the results presented here, a logical direction for future studies would involve assessment of the efficacy of both established and novel therapeutic approaches to PTSD and depression in patients with traumatic brain injury. Given the substantial burden of TBI, PTSD, and depression in Military service members and veterans who have volunteered for deployment to war zones, maximal efforts to improve outcomes are warranted.

Strengths of this study include the use of a prospective, observational, longitudinal cohort design; enrollment of all combat-deployed, active-duty US military; the inclusion of subjects with both blast-related and non-blast-related concussive TBI; the assessment of both blast-exposed and non-blast-exposed combat-deployed controls; the incorporation of both medically-evacuated and non-medically evacuated casualties; and the comparison of four independent cohorts of subjects across all branches of the military.

Nonetheless, there are several limitations that should be acknowledged: 1) unknown diagnostic accuracy for concussive TBI in the absence of an objective standard, 2) self-report for several of the key outcome measures including overall disability, 3) heterogeneous treatment across centers in theater and in the US after injury, 4) single time point for most assessments, 5) incomplete assessment of combat exposure severity, 6) no objective markers of the severity of initial injury [though initial MACE scores showed no trends over time], 7) possible unmeasured covariates that differ between groups, and 8) lack of long-term follow-up. It is unclear whether 6-12 month outcomes are truly representative of long term function or quality of life(39-43). Studies are currently underway to explore >5 year outcomes in these military concussive TBI cohorts.

Based on these data, it appears that the severity of PTSD and depression are strongly linked to overall outcomes following concussive TBI in US service members. However, the direction of causality cannot be determined from the current results. In our view, the most likely scenario is that concussive TBI along with the trauma-associated psychopathology (i.e. PTSD, depression) that accompanies deployment in a war zone interact in a synergistic fashion to worsen outcomes; TBI may damage the brain's emotional regulation circuitry and the trauma-associated psychopathology may interfere with recovery from TBI. However, it is also possible that the overall TBI severity is the primary driver of both overall outcomes and trauma-associated psychopathology. A third alternative is that the most stressful wartime events that caused the most persistent and severe PTSD and depression also caused acute amnesia or transient changes in awareness, and were therefore incorrectly labeled as concussive TBI. Clearly, future studies involving objective measures of primary brain injury severity and careful anatomical delineation of the relevant brain circuitry involved in emotional regulation will be required to address these alternatives.

METHODS

Study Design: Analysis of 4 prospective, observational, longitudinal cohort studies.

Subjects: We screened a total of 1105 subjects between 2008 and 2013 across four cohorts and enrolled a total of 591 subjects, 347 of whom completed follow up 6-12 months later at Washington University in Saint Louis (**Fig. 1**). The first 3 cohorts were enrolled at Landstuhl Regional Medical Center (LRMC) following medical evacuation from theater (Study 1-3). LRMC is the primary Role 4 evacuation hub for all medically evacuated casualties originating from Iraq and Afghanistan. Study 1 cohort was enrolled from November 2008 to August 2009 and accepted patients 0-90 days post-injury. Study 2 cohort was enrolled from September 2010 to March 2011 and accepted patients 0-30 days post-injury. Study 3 cohort was enrolled from October 2010 to May 2013 and accepted patients 0-30 days post-injury. Study 4 cohort was enrolled at Kandahar Air Field and Camp Leatherneck in Afghanistan from March to September 2012 and accepted patients 0-7 days post-injury who remained in theater. Reasons for non-enrollment included contraindications to study procedures (399), refusal to participate (99), inability to follow up (5), interference with medical care (5), and other (6).

Four groups of subjects were enrolled:

-Blast Control: Subjects with blast exposure but without clinical evidence of resultant TBI

-Non-Blast Control: Subjects without blast exposure and without TBI

-Blast+impact TBI: Subjects with blast-plus-impact concussive TBI.

-Non-blast TBI: Subjects with non-blast-related concussive TBI (i.e. TBI from mechanisms other than blast)

Inclusion criteria across cohorts for both the blast+impact and non-blast concussive TBI groups were as follows: 1A) a positive screen for TBI at LRMC based on standard US military clinical criteria(44) including self-report of blast exposure or non-blast mechanism such as blunt trauma resulting in loss of consciousness, amnesia for the event, or change in neurological status, for studies 1-3 or, 1B) a clinical diagnosis of TBI in

Afghanistan based on the criteria from the American Congress of Rehabilitation 1993, for study 4, 2) TBI from blast or non-blast mechanisms of injury within the specified time of enrollment, 3) US military service member, 4) ability to provide informed consent in person, 5) no contraindications to MRI such as retained metallic fragments, 6) no pre-deployment history of moderate to severe TBI based on Department of Defense criteria, 7) no pre-deployment history of major psychiatric disorder, 8) agreement to communicate by telephone or email monthly for 6-12 months and then travel to Washington University for in-person follow-up. Inclusion criteria for the control groups were the same except for a negative diagnosis of TBI. The requirement for in-person informed consent made more severe TBI patients typically not eligible and none were enrolled. No intracranial abnormalities were detected on non-contrast head CT. Thus, all TBI subjects met the DoD criteria for uncomplicated 'mild'/ concussive TBI.

For the control groups in the LRMC cohorts who were medically evacuated, gastrointestinal, dermatological, and women's health reasons were the main diagnoses. Orthopedic injuries from non-combat events such as broken bones resulting from recreational sports on time off or work-related accidents also comprised a subset of this population. The control group from Afghanistan mostly included onsite personnel who volunteered to participate in the study. A small number of controls enrolled in Afghanistan also had minor orthopedic injuries from non-combat events that did not require medical evacuation to LRMC. All clinical histories from the controls indicated no current or previous diagnoses of TBI. The blast control groups endorsed previous history of blast exposure but were found not to have had TBI following a clinical evaluation for possible brain injury at LRMC.

For the blast TBI groups across all of the cohorts, all available clinical histories indicated blast exposure plus another mechanism of head injury such as a fall, motor vehicle crash, or being struck by a blunt object. None suffered an isolated blast injury. The mechanisms of injury for the non-blast TBI group were primarily falls, motor vehicle crashes, or being struck by a blunt object that did not involve blast exposure. For both the

blast and non-blast TBI groups, clinical histories indicated a change in the level of consciousness or loss of consciousness for at most a few minutes and post-traumatic amnesia for less than 24 hours.

All clinical histories were verified by study personnel taking additional clinical history and reviewing medical records. All screening-based identifications of TBI were confirmed; none that screened positive for TBI were determined not to have had a TBI upon further inspection. Initial records of clinical status in TBI subjects using the Military Assessment of Concussion Exam (MACE)(44) were reviewed. This brief cognitive test assesses orientation, immediate verbal memory, concentration, and short term delayed verbal memory.

Clinical Assessments: Overall clinical outcome was assessed using the Glasgow Outcome Scale Extended (GOS-E)(45, 46). The GOS-E is scored from 1-8: 1=dead, 2=vegetative, 3-4=severe disability, 5-6=moderate disability, 7-8=good recovery. Moderate disability (GOS-E = 5-6) is defined as one or more of the following: 1) inability to work to previous capacity 2) inability to resume the majority of regular social and leisure activities outside the home 3) psychological problems which have frequently resulted in ongoing family disruption or disruption of friendships. For subjects with moderate disability, we further sub-categorized the disability as related to work, non-work, or both work and non-work. Severe disability (GOS-E = 3-4) is defined as reduced ability to perform activities of daily living such that supervision is required. Standardized, structured interviews were performed according to published guidelines(45).

In-person clinical evaluations at Washington University included a neurobehavioral assessment, neuropsychological test battery, and psychiatric evaluation. The neurobehavioral assessment involved a structured exam and interview designed for TBI patients (Neurobehavioral Rating Scale-Revised)(47), scored using a previously published 5 sub-domain model(48). The neuropsychological test battery consisted of the Conner's Continuous Performance Test II(49), a computer-based assessment of attention, impulsivity, reaction time, and vigilance; the California Verbal Learning Test II(50), an assessment of verbal declarative memory; the 25 hole grooved pegboard test(51), an assessment of upper extremity motor speed and coordination; a timed 25 foot walk; the Trail Making test(52), an assessment of visual scanning, coordination and mental flexibility;

the Controlled Oral Word Association test(53), an assessment of verbal fluency; and the Wechsler Test of Adult Reading(54), an estimate of pre-injury verbal intelligence. A relatively easy forced choice test embedded in the California Verbal Learning Test was used to assess adequacy of effort. 5 subjects, all from study 3, were disqualified for either poor effort or apparent malingering. The psychiatric evaluation included the Clinician-Administered PTSD Scale for DSM-IV (CAPS)(55) and the Montgomery-Asberg Depression Rating Scale (MADRS)(56). The CAPS was scored using standard scoring rules from the Blake et al, National Center for Post-traumatic Stress Disorder, July 1998 revision.

The 6-12 month follow-up evaluations involved approximately 5 hours of in-person assessments. The standardized neurological exam and interview required approximately 1 hour per subject. The psychiatric assessments required approximately 2 hours per subject, and the neuropsychological battery required approximately 2 hours per subject. Subjects took all medications as prescribed by their clinical providers. All tests were performed between 9 am and 5 pm in private, quiet, well-lighted rooms. All examiners were blinded to other clinical information, though in the course of the interviews it often became clear whether the subjects were in the TBI or control group based off their endorsements of prior events. All examiners were clinicians who underwent standardized training in administering the assessments.

Safety and Data Monitoring: Subjects were assigned a random 4 digit code number to protect confidentiality and all research data was identified by code number only. A board certified psychiatrist (E. Nelson) was immediately available in case the CAPS examination exacerbated PTSD symptoms. No exacerbations requiring medical intervention occurred, though additional support from study staff was required on several occasions.

For clinical evaluations, the principal investigator audited 1 in 10 randomly selected subjects' data sets to ensure that data was scored and entered correctly. These audits revealed only minor discrepancies in scoring criteria which were then corrected across the entire cohort of subjects.

Statistical Analyses: Data were analyzed using Statistica 12.0 (Statsoft Inc). The normal distribution of each continuous variable was assessed using the Shapiro-Wilk test. For normally distributed variables, Analysis of Variance, Analysis of Covariance and Student's t tests were used to compare groups. For non-normally distributed variables, Kruskal-Wallis Tests and Mann-Whitney U tests were used. We pre-specified the hypothesis that concussive TBI subjects would have worse outcomes than controls. One-sided tests were used when hypotheses were pre-specified, and two-sided tests were used otherwise. Uncorrected p-values have been reported, but only considered significant if $p<0.05$ after Bonferroni or Dunn correction for multiple comparisons.

Following Dunn's correction for multiple comparisons, there were no significant differences in GOS-E within comparable sub-groups of subjects across studies. Therefore, the data was combined into the following three groups for additional analysis: Non-blast control, blast control, concussive TBI. For ANCOVA and generalized linear models there were too few officers (8 total) or females (10 total) for accurate statistical assessment, so the analyses were limited to enlisted males.

To determine the number of neuropsychological tests expected to be abnormal by chance, the binomial distribution was used with $p=0.02275$ for the ($n=13$) neuropsychological variables examined. Prior to this analysis, all neuropsychological variables were confirmed to be statistically independent as is required by the assumptions of this approach. There were no significant differences in the number of subjects with abnormal neuropsychological test performance in 2 or more neuropsychological assessments between evacuated TBI subjects, non-evacuated TBI subjects, and blast control subjects.

Logistic regression modeling was utilized to explore the relationship between a dichotomized measure of clinical outcome (GOS-E) and the demographic and clinical measures collected 6-12 months post injury. For logistic regression, the Statistica 12.0 'generalized linear/nonlinear model building' algorithm was used with the selection of the 'logit' link function for logistic regression. The algorithm generated a distinct model for each possible subset of demographic data and quantitative measures of specific symptoms and impairments. Models

were then ranked by Akaike information criterion(57), which penalizes models with larger numbers of parameters to discourage overfitting. Many subjects injured after the issuance of the DTM were evacuated from theater as late as May 2013, so the enrollment site distinction and date of injury were not redundant.

Study approval: The research protocol was approved by the Human Research Protection Office at Washington University, the Institutional Review Board at Brooke Army Medical Center, the Clinical Investigation Regulatory and Human Research Protection Offices of the U.S. Army Medical Research and Materiel Command and the Department of Defense Central Command Medical Research and Materiel Command Institutional Review Board. Written informed consent was obtained from all subjects in person at the time of enrollment; no surrogate consent was allowed. Competence to provide informed consent was assessed in a standardized fashion based on responses to questions regarding the purpose of the study, expected requirements for participation, and potential risks. Additional written consent was obtained from the subjects at the time of follow-up at Washington University. Active duty military subjects were not paid for participation, though travel expenses to Washington University were covered. Subjects not on active military duty status at the time of follow-up were paid $240 plus travel expenses for participation.

AUTHOR CONTRIBUTIONS

Designed research: CLM, ECN, NJW, ORA, SFF, DLB
Obtained funding: DLB
Enrolled subjects: CLM, LW, EK, TS, ORA, DJR
Supervised research: CLM, ECN, NJW, ORA, DJR, SFF, JSO, DZ, RF, DLB
Collected data: CLM, AMJ, LW, EK, TS, ECN, NJW, ORA, DJR, DLB
Analyzed data: CLM, ORA, DLB
Prepared manuscript and figures: CLM, DLB
Edited manuscript and figures: all authors.
The principal investigator and first author, DLB and CLM, take responsibility for the integrity of the data and analysis.

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of the Army, Department of the Navy, the Department of the Air Force, Department of Defense, or U.S. Government.

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TABLES

Table 1. Follow Up Participant Characteristics

Characteristic	Study 1		Study 2	Study 3				Study 4	
	Blast Control (n=18)	Blast TBI (n=47)	Blast TBI (n=32)	Non-blast Control (n=69)	Blast Control (n=27)	Non-blast TBI (n=29)	Blast TBI (n=53)	Non-blast Control (n=34)	Blast TBI (n=38)
Age in years: median (range)	32 (20-49)	26 (19-45)	24 (19-44)	31 (21-49)	34 (22-46)	28.5 (20-50)	26 (19-47)	28 (19-44)	26 (20-41)
Education in years: median (range)	13 (12-18)	12 (8-17)	12 (9-16)	14 (9-28)	13 (10-19)	14 (9-18)	12 (12-18)	15 (12-24)	13 (12-18)
Gender: no (%)									
Male	18 (100%)	47 (100%)	29 (91%)	63 (91%)	25 (93%)	26 (90%)	51 (96%)	27 (79%)	36 (95%)
Female	0	0	3 (9%)	6 (9%)	2 (7%)	3 (10%)	2 (4%)	7 (21%)	2 (5%)
Race/ethnicity: no (%) †									
White	15 (83%)	35 (74%)	22 (68%)	50 (73%)	20 (74%)	19 (60%)	40 (76%)	22 (65%)	29 (77%)
African American	2 (11%)	5 (11%)	5 (16%)	16 (23%)	4 (15%)	7 (27%)	4 (6%)	5 (15%)	2 (5%)
Hispanic/Latino	1 (6%)	2 (4%)	5 (16%)	3 (4%)	2 (7%)	3 (10%)	7 (14%)	7 (20%)	7 (18%)
Asian	0	5 (11%)	0	0	1 (4%)	1 (3%)	2 (4%)	0	0
Branch of Service: no (%)									
US Army	15 (83%)	42 (89%)	26 (81%)	55 (80%)	24 (89%)	26 (90%)	46 (90%)	13 (38%)	32 (84%)
US Air Force	2 (11%)	0	0	11 (16%)	0	2 (7%)	1 (2%)	2 (6%)	0
US Marine Corps	1 (6%)	5 (11%)	5 (16%)	3 (4%)	3 (11%)	1 (3%)	5 (6%)	3 (9%)	6 (16%)
US Navy	0	0	1 (3%)	0	0	0	1 (2%)	16 (47%)	0
Military Rank: no (%)									
Enlisted	16 (89%)	45 (96%)	32 (100%)	63 (91%)	24 (89%)	27 (93%)	52 (98%)	24 (71%)	35 (92%)
Officer	2 (11%)	2 (4%)	0	6 (9%)	3 (11%)	2 (7%)	1 (2%)	10 (29%)	3 (8%)
Theatre of Operation: no (%)									
Afghanistan	6 (33%)	28 (60%)	27 (84%)	55 (80%)	21 (78%)	18 (62%)	50 (94%)	34 (100%)	38 (100%)
Iraq	12 (69%)	19 (40%)	5 (16%)	14 (20%)	6 (22%)	11 (38%)	3 (6%)	0	0

Table 2. Comparison of TBI Service Members, Follow Up vs. No Follow Up											
Study		**Study 1**		**Study 2**		**Study 3**				**Study 4**	
Group		**Blast TBI (n=65)**		**Blast TBI (n=40)**		**Blast TBI (n=79)**		**Non-blast TBI (n=44)**		**Blast TBI (n=95) *50 Invited for Follow Up**	
Follow Up Status		Follow Up (n=47)	No Follow Up (n=18)	Follow Up (n=32)	No Follow Up (n=8)	Follow Up (n=53)	No Follow Up (n=26)	Follow Up (n=29)	No Follow Up (n=15)	Follow Up (n=38)	No Follow Up (n=57)
Age in years:	**median (range)**	26 (19-47)	25 (19-45)	24 (19 - 44)	24 (22 - 31)	26 (19-47)	24 (20 43)	28.5 (20-50)	24 (22-48)	26 (20-41)	25 (20-41)
Gender no (%)	**Male**	47 (100%)	18 (100%)	29 (91%)	8 (100%)	51 (96%)	24 (92.3%)	26 (86.7%)	14 (93.3%)	36 (95%)	57 (100%)
	Female	0	0	3 (9 %)	0	2 (4%)	2 (7.7%)	3 (13.3%)	1 (6.6%)	2 (5%)	0
Branch of Service no (%)	**US Army**	42 (89%)	16 (89%)	28 (88%)	6 (76%)	46 (89.8%)	20 (76.9%)	26 (90%)	10 (66.8%)	32 (84%)	47 (82%)
	US Air Force	0	0	0	0	1 (2%)	2 (7.7%)	2 (6.7%)	1 (6.6%)	0	0
	US Marine Corps	5 (11%)	2 (11%)	4 (12%)	1 (12%)	5 (6.1%)	4 (15.4%)	1 (3.3%)	3 (20%)	6 (16%)	9 (16%)
	US Navy	0	0	0	1 (12%)	1 (3.1%)	0	0	1 (6.6%)	0	1 (2%)
Military Rank no (%)	**Enlisted**	45 (96%)	18 (100%)	32 (100%)	8 (100%)	52 (98%)	25 (96.2%)	27 (93.3%)	15 (100%)	35 (92%)	54 (95%)
	Officer	2 (4%)	0	0	0	1 (2%)	1 (3.8%)	2 (6.7%)	0	3 (8%)	3 (5%)
MACE Exam Score	**median (range)**	25 (5 - 30)	25 (19 - 29)	25 (19 - 30)	25 (24 - 29)	26 (12 – 30)	25 (16-30)	26 (21-30)	26 (10-30)	24 (9-30)	24 (3 - 30)

Table 3. Control Service Member Characteristics, Follow Up vs. No Follow Up									
Study		**Study 1**		**Study 3**				**Study 4**	
Group		**Blast Control (n=21)**		**Blast Control (n=35)**		**Non-blast Control (97)**		**Non-Blast Control (101) *50 Invited for Follow Up**	
Follow Up Status		Follow Up (n=18)	No Follow Up (n= 3)	Follow Up (n=27)	No Follow Up (n=8)	Follow Up (n=69)	No Follow Up (n=28)	Follow Up (n=34)	No Follow Up (n=67)
Age in years:	**median (range)**	31 (21-49)	22 (20-23)	34 (22-46)	29 (20-39)	31 (21-49)	30(22-49)	28 (19-44)	27 (20-48)
Gender no (%)	**Male**	18 (100%)	2 (67%)	25 (92.3%)	6 (75%)	63 (91.3%)	24 (85.7%)	27 (79%)	52 (78%)
	Female	0	1 (33%)	2 (7.7%)	2 (25%)	6 (8.7%)	4 (14.3%)	7 (21%)	15 (22%)
Branch of Service no (%)	**US Army**	15 (83%)	3 (100%)	24 (88.5%)	6 (75%)	55 (79.7%)	25 (89.3%)	13 (38%)	26 (39%)
	US Air Force	2 (11%)	0	0	1 (12.5%)	11 (15.9 %)	3 (10.7%)	2 (6%)	10 (15%)
	US Marine Corps	1 (6%)	0	3 (11.5%)	1 (12.5%)	3 (4.3%)	0	3 (9%)	8 (12%)
	US Navy	0	0	0	0	0	0	16 (47%)	23 (34%)
Military Rank no (%)	**Enlisted**	16 (89%)	3 (100%)	24 (88.5%)	8 (100%)	63 (91.3%)	26 (92.9%)	24 (71%)	54 (81%)
	Officer	2 (11%)	0	3 (11.5%)	0	6 (8.7%)	2 (7.1%)	10 (29%)	13 (19%)

Note that no controls were enrolled in Study 2

Table 4. Neuropsychological Test Performance

Assessment	Study 1		Study 2	Study 3				Study 4	
	Blast Control (n=18)	Blast+Impact TBI (n=47)	Blast +Impact TBI (n=32)	Non Blast Control (n=69)	Blast Control (n=27)	Non Blast TBI (n=29)	Blast+Impact TBI (n=53)	Non Blast Control (n=33)	Blast+Impact TBI (n=38)
25-Foot Walk (seconds)*	5.18 ± 2.05	4.96 ± 1.02	4.65 ± 1.37	3.92 ± 0.82	4.22 ± 0.66	4.76 ± 1.16	4.59 ± 1.17	3.78 ± 0.60	4.23 ± 0.68
(Motor Strength, Balance, Coordination)									
Conners' Continuous Performance Test II									
Omission Errors (T-score):	54.49 ± 21.18	51.39 ± 12.56	75.67 ± 64.71	48.29 ± 12.17	47.45 ± 7.51	53.30 ± 15.11	56.06 ± 19.8	48.85 ± 10.51	60.41 ± 28.13
(*Attention Lapses*)									
Commission Errors (T-score):	50.92 ± 10.54	51.73 ± 9.64	55.36 ± 8.85	50.40 ± 10.60	50.02 ± 8.19	52.46 ± 9.81	54.05 ± 10.6	53.83 ± 11.03	54.69 ± 10.16
(*Impulsivity*)									
Hit Rate (T-score):	49.4 ± 11.22	47.69 ± 9.04	47.88 ± 12.80	48.94 ± 11.72	48.98 ± 8.67	52.10 ± 12.22	47.83 ± 8.63	46.06 ± 9.88	50.81 ± 10.33
(*Reaction Time*)									
Hit Rate Block Change (T-score):	52.62 ± 10.29	52.17 ± 10.74	49.92 ± 13.73	52.05 ± 10.62	48.01 ± 8.82	51.64 ± 13.75	48.73 ± 12.0	48.67 ± 5.56	54.69 ± 13.43
(*Sustained Vigilance)*									
Wechsler Test of Adult Reading (Standard Score)	97.56 ± 12.56	98.3 ± 11.74	100.09 ± 10.48	102.88 ± 14.55	100.56 ± 10.99	98.52 ± 11.10	99.49 ± 11.66	105.41 ± 10.58	99.03 ± 12.50
(Estimate of Pre-injury Verbal Intelligence)									
California Verbal Learning Test II									
Long-Delay Free Recall (Standard Score)	0 ± 0.89	-0.13 ± 0.94	-0.33 ± 1.31	-0.17 ± 1.10	-0.15 ± 0.95	-0.32 ± 1.27	-0.58 ± 1.21	0.15 ± 1.28	-0.57 ± 0.92
(Verbal Memory)									
Total Intrusions (Standard Score)	0.44 ± 1.45	0.15 ± 1.04	0.28 ± 1.10	0.22 ± 1.00	0.22 ± 0.95	0.52 ± 1.42	0.45 ± 1.38	0.14 ± 0.84	0.50 ± 1.22
(Falsely Recalled Items)									
List B vs. Trial 1 List A (Standard Score)	0.11 ± 1.13	-0.34 ± 1.11	-0.23 ± 1.16	0.08 ± 0.87	-0.15 ± 0.89	0.58 ± 1.03	-0.16 ± 1.12	0.00 ± 1.05	-0.12 ± 0.90
(Proactive Memory Interference)									
Grooved Pegboard*									
(Motor Speed & Coordination)									
Average Dominant & Non-Dominant Time (seconds)	80.94 ± 11.54	77.31 ± 12.65	78.72 ± 14.28	69.03 ± 17.7	69.04 ± 10.56	75.84 ± 15.85	75.54 ± 15.52	67.68 ± 10.34	71.63 ± 7.74
Trail Making Test									
Trails A time (seconds)	24.78 ± 5.86	27.28 ± 10.54	28.02 ± 11.28	22.10 ± 8.61	24.26 ± 7.41	26.57 ± 14.10	28.5 ± 16.69	23.24 ± 7.65	23.6 ± 7.08
(Visual Scanning, Coordination)									
Trails B time (seconds)	59.56 ± 15.80	66.79 ± 22.53	63.06 ± 19.01	57.12 ± 24.77	57.00 ± 14.97	67.52 ± 31.28	61.19 ± 21.40	55.38 ± 18.65	64.43 ± 23.89
(Trails A + Mental Flexibility)									
Controlled Oral Word Association*	34.33 ± 7.35	35.91 ± 9.31	34.19 ± 9.53	42.1 ± 10.18	40.37 ± 9.05	37.62 ± 9.98	37.75 ± 9.30	42.82 ± 9.61	41.45 ± 11.47
Total Score: *(Verbal Fluency)*									

TABLE 4. Neuropsychological Test Performance.

All data reported as mean ± standard deviation.

* Significant group differences by Kruskal-Wallis ANOVA after Bonferroni correction for multiple comparisons.

FIGURES AND LEGENDS

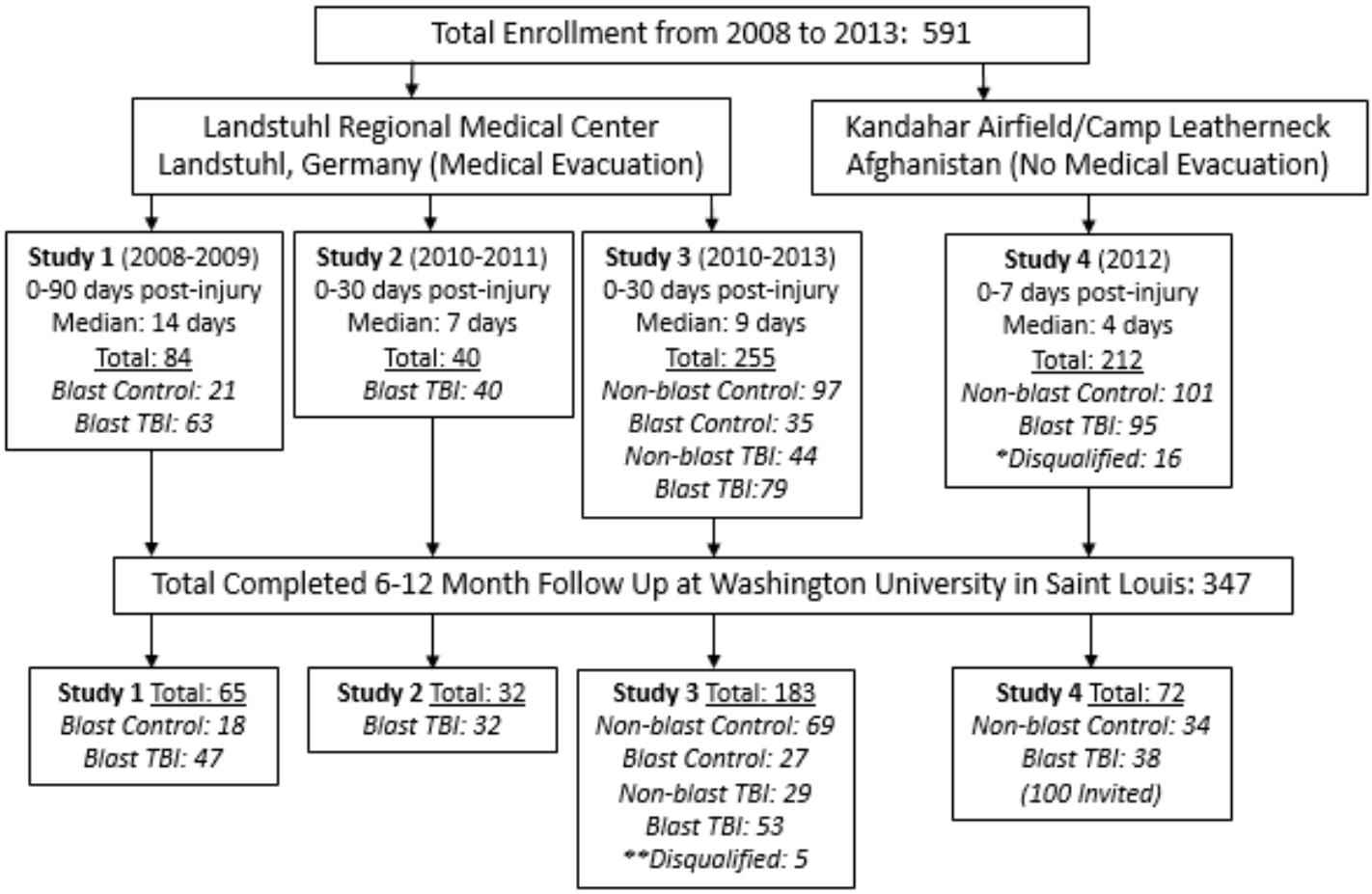


FIG 1. Consort Diagram of Enrollment.

*Subjects disqualified for poor performance on the Test of Memory Malingering and/or substantial artifacts on MRI; a criteria of the study.

**Subjects disqualified at follow up for apparent malingering and/or erratic performance on clinical evaluations.

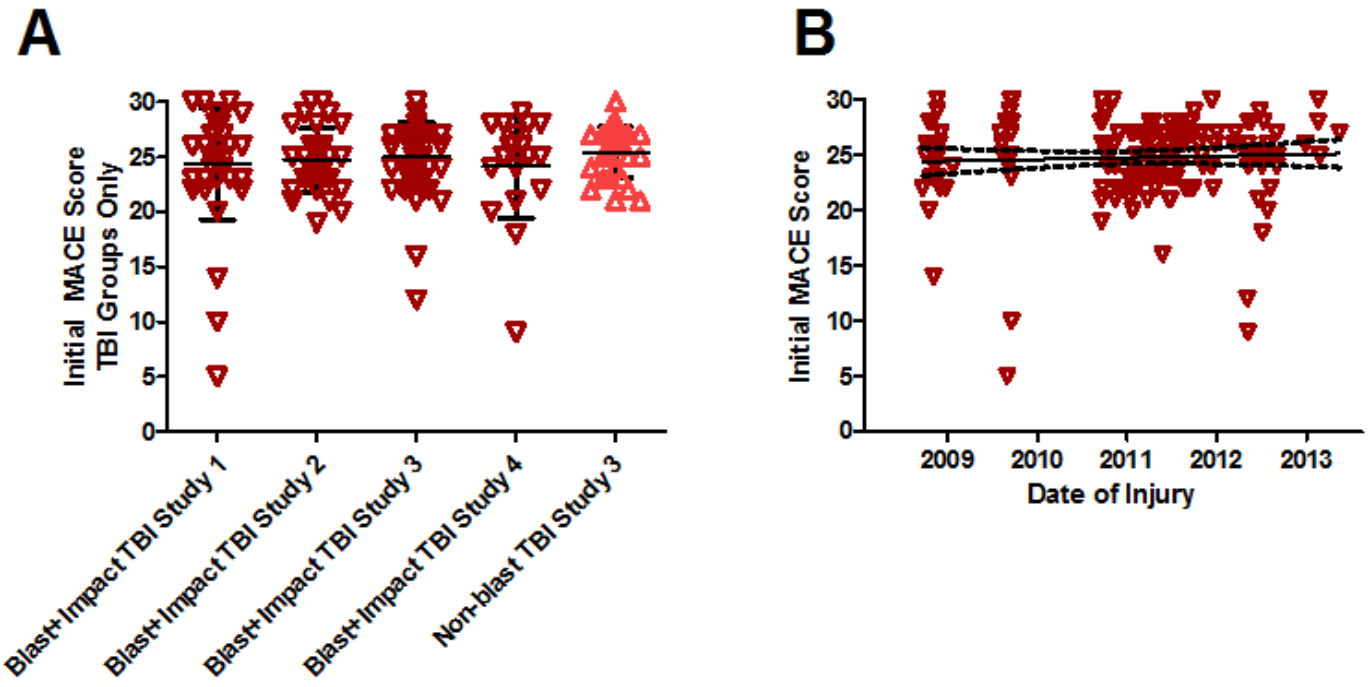


FIG. 2. Military Assessment of Concussion Evaluation (MACE). Lower scores indicate greater concussion impairment (Max 30, Symptomatic defined as below 25 on any assessment(44)). **A.** No difference in MACE between cohorts (p=0.87 Kruskal Wallis ANOVA). **B.** No trends in MACE as a function of date of injury (p=0.52, linear regression). Note that MACE was not performed in controls.

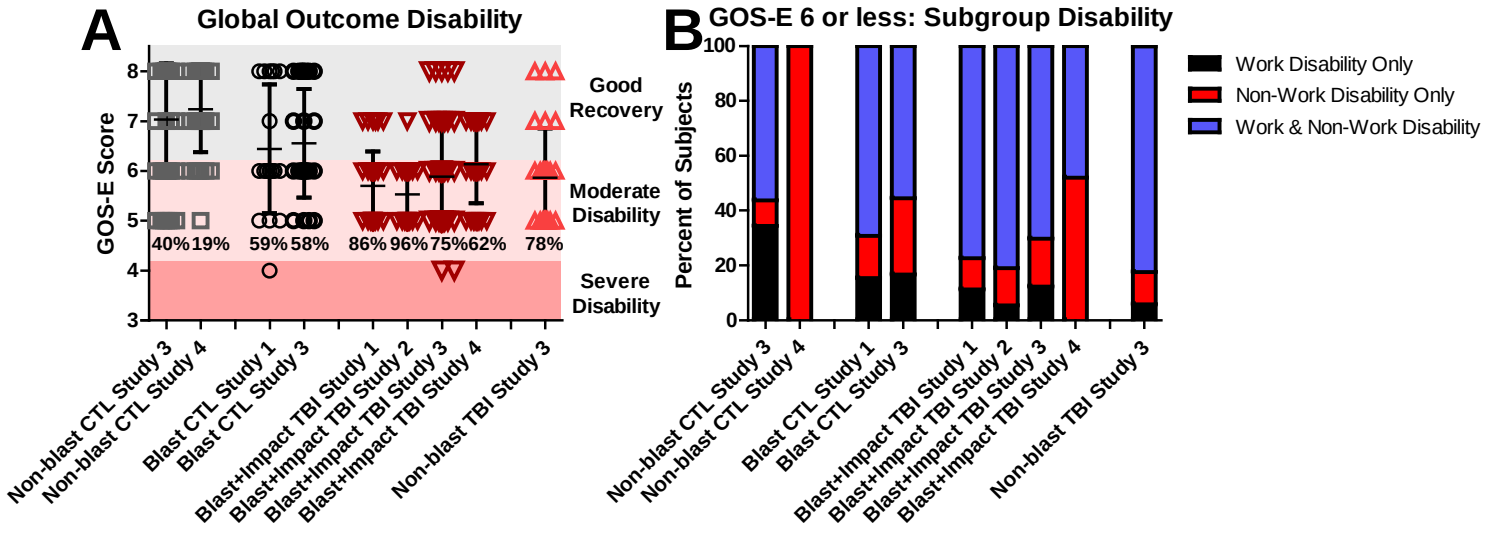


FIG. 3. Global Outcome

FIG. 3. Global Outcome. A. Glasgow Outcome Scale – Extended (GOS-E). Percent of service members with moderate to severe disability are reported under each study group on the graph. **B.** Subgroup disability for service members with GOS-E score of 6 or less.

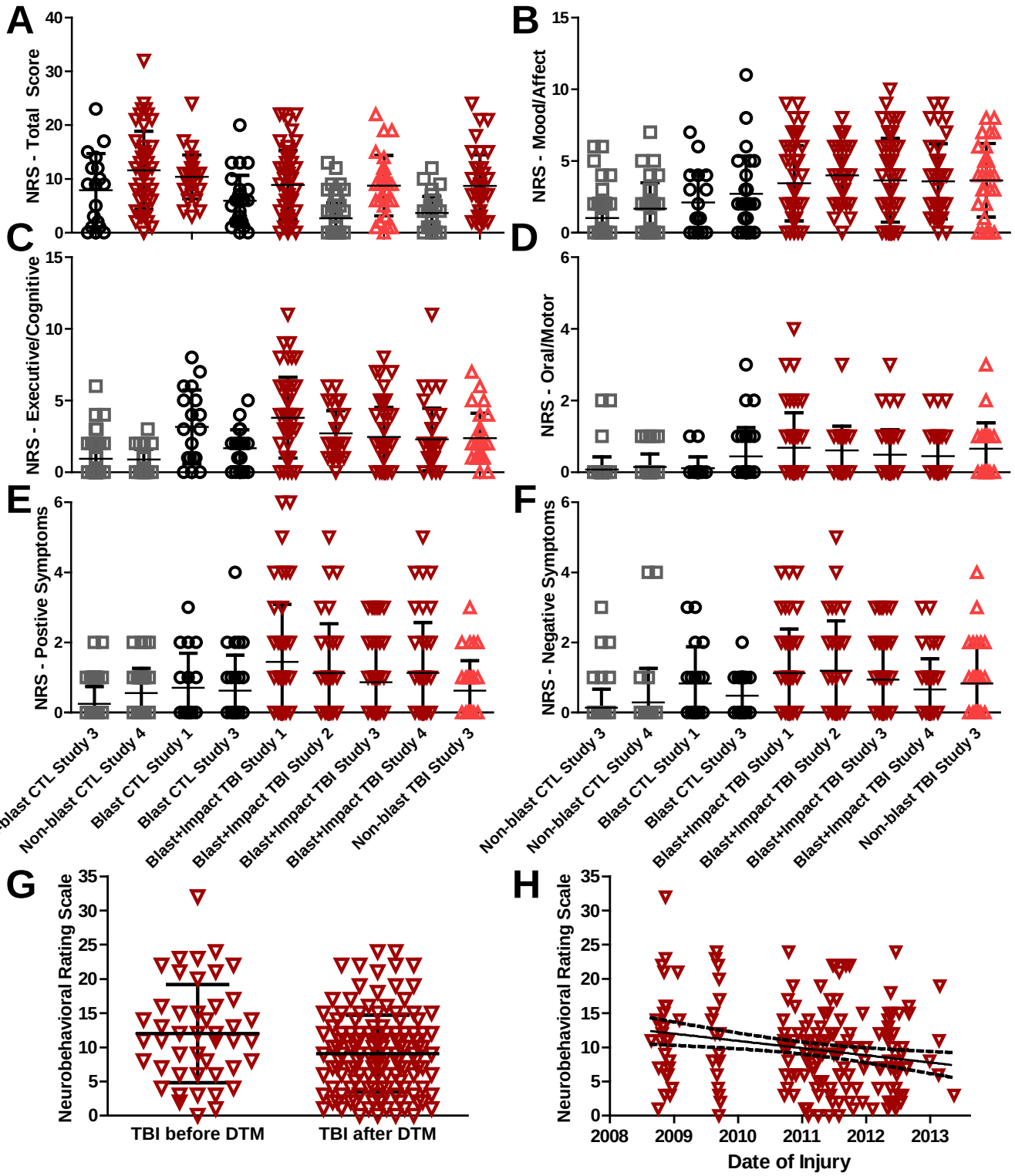


FIG. 4. Neurobehavioral Rating Scale and Sub-Domains

FIG. 4. Neurobehavioral Rating Scale and Sub-Domains.

A. Neurobehavioral outcome assessed using the Neurological Rating Scale-Revised (NRS) Total Score: (Max 87, higher scores indicate worse outcomes). Results assessed 6-12 months after enrollment. **B**..Mood/affect domain (Max 15). **C.** Executive/Cognitive domain (Max 24). **D.** Oral/motor domain (Max 12). **E.** Positive Symptoms domain (Max 21). **F.** Negative Symptoms domain (Max 12). Higher scores on all of the measures indicate worse impairment. All $p<0.0001$, Kruskal Wallis ANOVA. **G.** Worse neurobehavioral outcomes before the issuance of the DTM on 6/21/10 compared to afterwards in concussive TBI subjects ($p=0.017$, Mann-Whitney U test, $p=0.057$ ANCOVA including covariates). **H.** Trend towards reduced neurobehavioral impairment over time in concussive TBI subjects ($p=0.0037$ linear regression, $p=0.08$ generalized linear model including covariates).

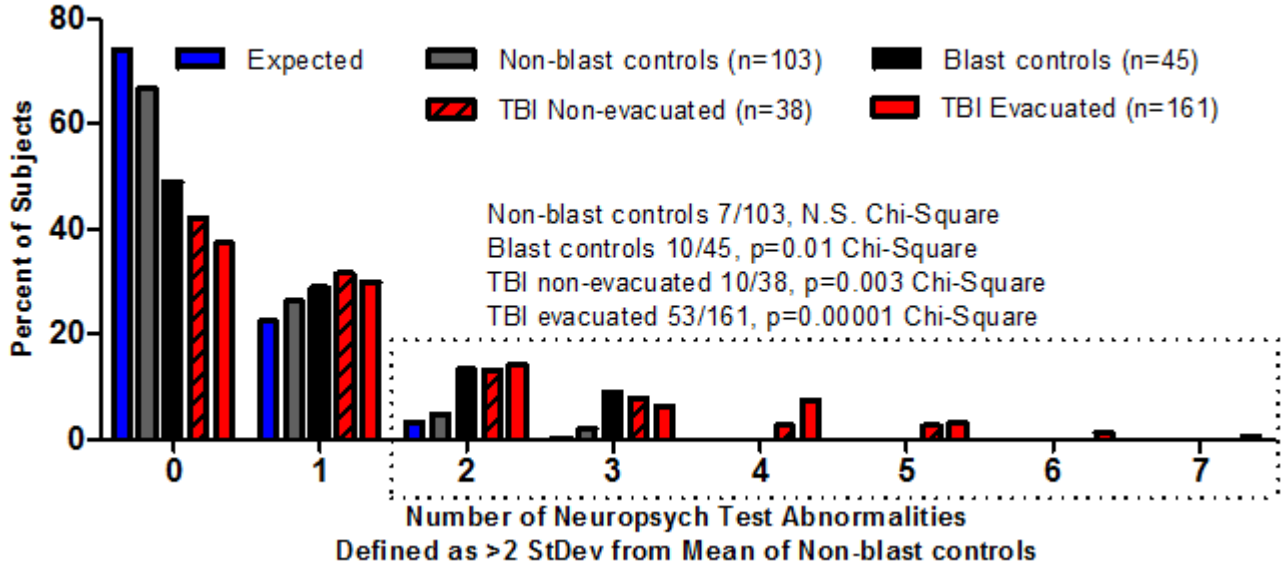


FIG. 5. Neuropsychological testing abnormalities. The number of subjects with neuropsychological test abnormalities are displayed by group in comparison to what would be expected by chance (blue bars). Percent of subjects is displayed to account for the differences in the number of subjects in each group. Dotted box indicates the group of subjects who had poor performance on 2 or more of the 13 neuropsychological variables.

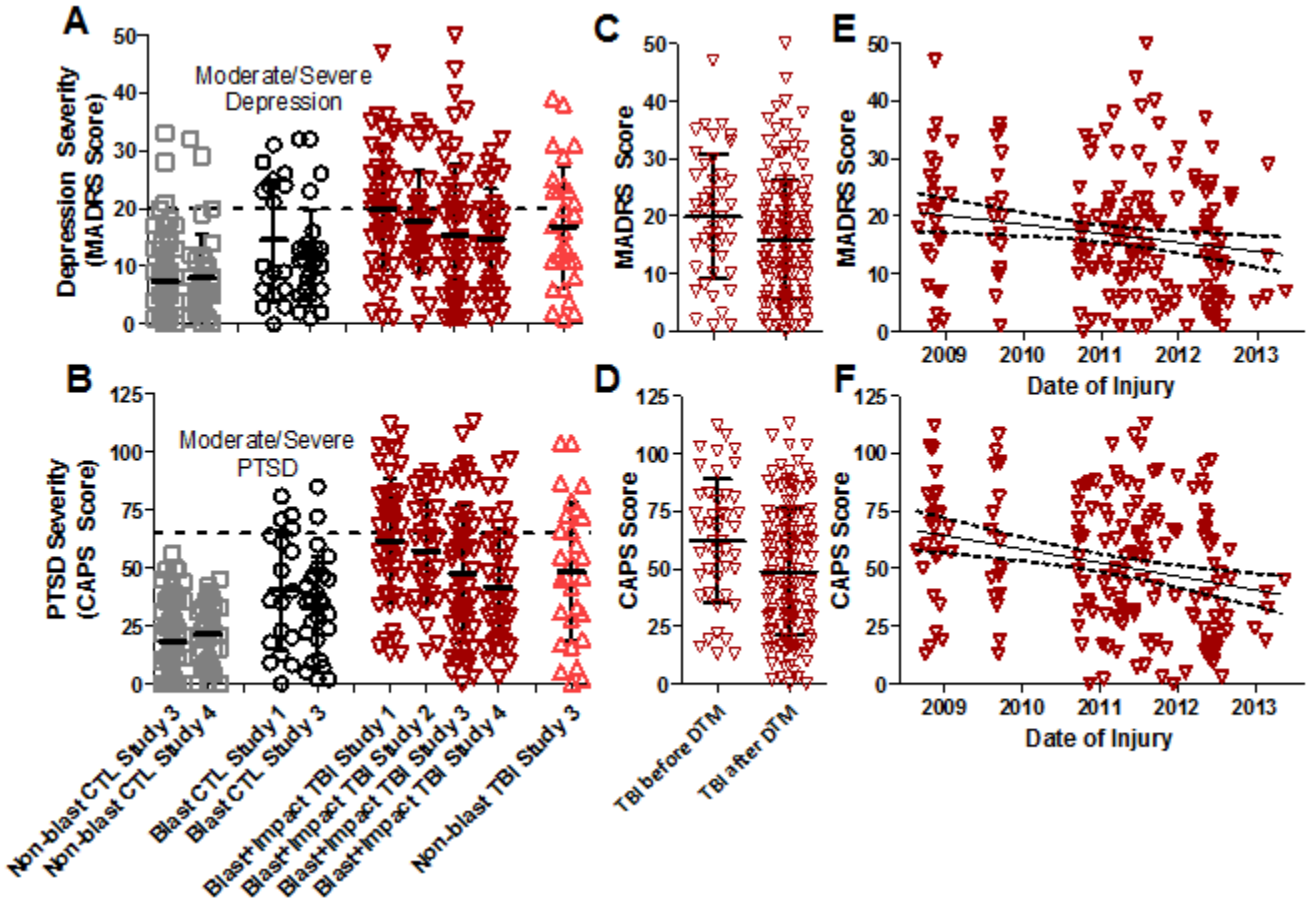


FIG. 6. PSTD and Depression Severity. A. Depression severity assessed by the Montgomery Asberg depression rating scale (MADRS) (Max 60). **B.** PTSD severity assessed by the Clinician administered PTSD scale for DSM IV (CAPS) (Max 136). Dotted lines indicate the threshold for moderate to severe symptomatology for each evaluation. **C.** Worse depression before the issuance of the DTM compared to afterwards in concussive TBI subjects (p=0.02, Mann-Whitney U test, p=0.12 ANCOVA including covariates). **D.** Worse PTSD before the issuance of the DTM compared to afterwards in concussive TBI subjects (p=0.006, Mann-Whitney U test, p=0.07 ANCOVA including covariates). **E.** Trend towards reduced depression over time in concussive TBI subjects (p=0.012 linear regression, p=0.15 generalized linear model). **F.** Statistically significant reduction in PTSD over time in concussive TBI subjects (p=0.00037 linear regression, p=0.03 generalized linear model including covariates).

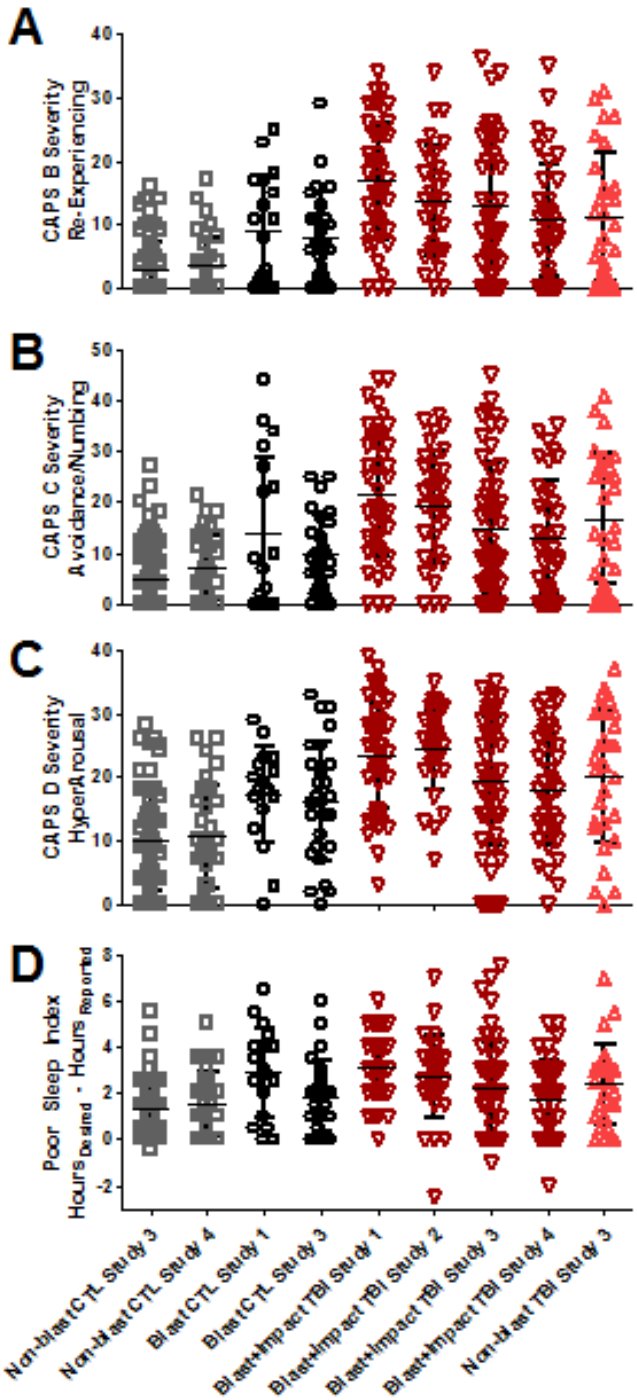
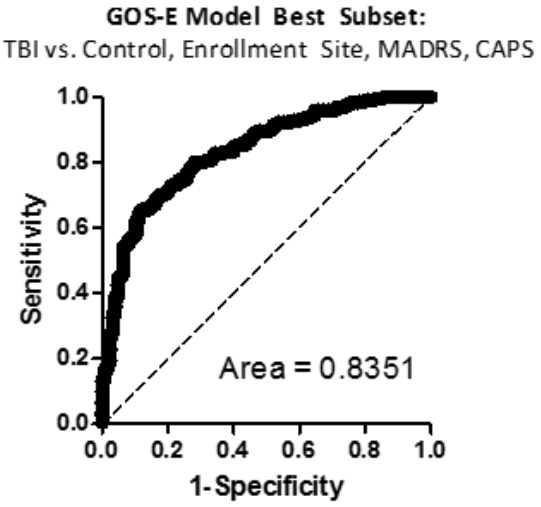


FIG. 7. Sub-Domains of the Clinician Administered PTSD Scale (CAPS) for DSM IV. A. CAPS B Severity – Re-experiencing (Max 40). **B**. CAPS C Severity – Avoidance and Numbing (Max 56). **C**. CAPS D Severity – Increased Arousal and hypervigilance (Max 40). **D.** Poor sleep index, defined as the self-reported number of desired hours of sleep minus the number of hours reported taken from subsection D-1 of the CAPS. Higher scores on all of the measures indicate worse impairment. All $p<0.0001$, Kruskal Wallis ANOVA.



Best Fit Model - Dichotomized GOSE				
Overall model : AIC 337.01, Likelihood ratio Chi square: 125.66				
Parameter	**Odds Ratio**	**95% Confidence Interval**	**P-value**	**Worse Outcome**
TBI /Control	1.73	(1.30 : 2.29)	0.0001	TBI
Enrollment Site: Medical-Evacuation vs. Non-Medically-Evacuated	1.76	(1.28 : 2.43)	0.0005	Medical-Evacuation
MADRS (Depression Symptoms)	1.07	(1.01 : 1.12)	0.0133	Higher Score
CAPS (PTSD Symptoms)	1.02	(1.00 : 1.04)	0.0223	Higher Score

FIG. 8. Correlates of Global Outcome. A. Receiver-operator curve and parameter table for best fit logistic regression model of overall disability, defined by the dichotomized GOS-E with 7 or 8 categorized as good outcome and GOS-E 6 or below categorized as disabled. The best model by Akaike information criterion contained the CAPS score (PTSD severity), MADRS score (Depression severity), injury group distinction (TBI vs. Control), and enrollment site distinction (subjects requiring medical evacuation vs. those that did not require medical evacuation).